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Review Article

Monitoring for incipient dreissenid mussel invasions using environmental DNA and RNA: review of current state of knowledge and considerations for implementation

Madeleine Malmfeldt[1], Richard Lance[1]

1 *Environmental Laboratory, US Army Engineer Research and Development Center, 3909 Halls Ferry Road, Vicksburg, MS, 39180, USA*
Corresponding author: Madeleine Malmfeldt (madeleine.p.malmfeldt@erdc.dren.mil)

Abstract

The establishment of populations of zebra and quagga mussel (*Dreissena polymorpha* and *D. bugensis*) is a continuing challenge for aquatic ecosystems and natural resource managers. Monitoring aquatic systems for the environmental DNA and RNA (eNA, collectively) of *Dreissena* is an effective means for detecting early-stage invasions by these species. There are several factors that should be considered when designing such a monitoring programme, many of which can be informed by existing literature in the eNA field, including several *Dreissena* eNA studies. We identified seasonal timing of *Dreissena* surveys to be a critical consideration, as detection will be maximised during spawning and veliger dispersal, but precision in identifying source locales may be greatest outside of this season. While many sampling decisions are specific to individual study locations, sampling from the surface and, in the case of lotic reaches, from areas of high energy, such as riffles, are likely to increase detection if *Dreissena* are present. Likewise, consideration of the particulars of the local environment, including seasonal wind and current patterns, will likely allow for more strategic sampling of *Dreissena* eNA and more informed inferences from survey outcomes. We also identified several future research areas that would better inform *Dreissena* eNA studies, such as behaviour of the transportation of eNA, specifically for *Dreissena*, in both lentic and lotic environments and the possibility of using genetic eNA techniques to indicate periods of *Dreissena* spawning and veliger presence.

Key words: Biosurveillance, bivalves, *Dreissena*, eDNA, eRNA, invasive species, Mollusca, quagga mussel, zebra mussel

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Introduction

Background

The objective of this literature review is to synthesise the various factors that should be considered when establishing an environmental DNA or RNA (eNA) surveillance programme for *Dreissena* and to provide some basic guidelines for implementing such a programme. While the guidelines we provide give best practice recommendations, there is still a high chance of non-detections when the species is rare or spread thinly throughout the system. Our recommendations seek to maximise detections under conditions such as those. Though both species may be found in estuaries and other brackish water habitats, our review focuses on freshwater systems, which are the dominant habitat for *Dreissena* in North America.

Aquatic invasive species often represent substantial economic and ecological challenges in affected waterways (Bij de Vaate et al. 2013). This reality has rarely been so substantively demonstrated than with two related bivalves, the zebra mussel (*Dreissena polymorpha*) and quagga mussel (*D. bugensis*). Both species are native to the Black Sea region of eastern Europe and western Asia, but have spread to many areas of western Europe and North America. First established in the North American Great Lakes in the mid-1980s, by 2024 *Dreissena* mussels could be found in at least 450 different hydrological unit sub-basins across 41 States and provinces of the United States (US), Canada (CA) and Mexico (MX) (Karatayev et al. 2015; U.S. Geological Survey 2024). For the purposes of this paper, the terms *Dreissena* and dreissenid are intended to refer solely to zebra and quagga mussels and not to any other species of *Dreissena*, none of which has emerged as problematic invasive species (reviewed in Van der Velde et al. (2010); Stepien et al. (2013)).

Dreissena species are well known for forming dense, well–anchored colonies that can clog and otherwise foul submerged pipes and other important components of waterway infrastructure (Higgins and Zanden 2010; Nalepa and Schloesser 2013). A recent review (Haubrock et al. 2022) placed the cumulative global cost of invasive Dreissenidae (primarily *D. polymorpha* and *D. bugensis*) from 1980–2020 at more than $51 billion (USD, 2017 value). The spread of these species is in large part due to their being transported overland or between inland waterbodies on recreational or commercial boating traffic. Both adults and larvae become ensconced in components of vessels that are difficult to monitor, such as motor cooling chambers, ballast tanks or bags or in bilge reservoirs (Dalton and Cottrell 2013). After transportation between waterbodies, *Dreissena* establish large, highly dense populations that may substantially reduce food availability for other filter-feeding organisms, such as native mussels, a situation which provides a competitive advantage for *Dreissena* and depresses local populations of native planktivorous taxa (Haag and Garton 1992; Higgins and Zanden 2010; Strayer and Malcom 2018).

As is generally the case with invasive species, detection of invasions during the initial or early stages of population establishment is expected to improve chances for effective eradication or control of those populations. However, the detection of invasive species at low abundances or densities typical of early establishments can be challenging. Monitoring of at–risk waterbodies, paired with prevention strategies like watercraft inspections, is the foremost approach for limiting the spread of *Dreissena* into new territories. Widespread, continual vigilance for these species at nearly any scale is a significant challenge.

Over the last decade and a half, environmental DNA (eDNA) surveys have been shown to be an efficient and effective means for detecting the presence of invasive species (Ficetola et al. 2008; Lodge et al. 2012; Morisette et al. 2021), including *Dreissena* mussels (Lance and Carr 2012; Peñarrubia et al. 2016; Blackman et al. 2020). More recently, environmental RNA (eRNA) has likewise emerged as a viable environmental tracer for biotic inspections, surveys and monitoring (Pochon et al. 2017; Wood et al. 2020; Marshall et al. 2021). As eDNA and eRNA (collectively, eNA) and most eNA–bearing materials will disperse from the source (an individual or group of individual organisms) in an aquatic environment, eNA provides a detectable signal of organism presence that is not limited by direct observations. Increased likelihood of detection makes eNA an attractive option for monitoring *Dreissena* invasions.

There are many different methodological alternatives for capture, processing and assaying water samples for eNA. The most used analysis methods are quantitative polymerase chain reaction (qPCR), digital polymerase chain reaction (dPCR) and metabarcoding via next generation sequencing. By and large, all conventional eNA detection platforms and approaches have been shown, when paired with well–optimised primers and probes, to be capable of detecting even small concentrations of eNA (Doi et al. 2015; Johnsen et al. 2020; Klymus et al. 2020; Shelton et al. 2023; Stelzer et al. 2024). Less-established methods include point-of-use field amplification that can provide more rapid, if less sensitive, results of *Dreissena* presence (Williams et al. 2017). Given the advanced state of eNA detection capabilities in general and specifically for *Dreissena* (Feist and Lance 2021), we will not focus our review on the analytical methods aspect of *Dreissena* eNA monitoring. Instead, we will explore critical questions about how, when and where to sample to maximise detection of eNA arising from small populations of *Dreissena* whose exact locations are unknown and which may represent an early stage of invasion. Key to understanding these facets of *Dreissena* eNA detection is a robust knowledge of the dynamics of *Dreissena* eNA in a system.

Barnes and Turner (2016) describe four major factors that influence the natural dynamics of eDNA: origin, state, fate and transport, (i.e. the ecology of eNA). While the ecology of eNA will vary amongst individual systems, there are some basic commonalities across all systems or within broad subsets of systems, that will likely be useful when designing strategic eNA monitoring for *Dreissena*. The origins of eNA are manifold, but some of the most universally applicable include sloughed gut epithelial cells passed with biological wastes, excreted organismal remains following predation, sloughed surface epithelial cells (e.g. skin, gills), blood and other material released following injury and wounding, decaying cells from deceased organisms and gametes broadcast or deposited in the external environment. In some cases, such as with *Dreissena*, minute, intact juveniles might also be considered a form of eNA because they are likely to be captured in water samples when present (Pawlowski et al. 2020). Multiple states, forms or classes of eNA can exist in the environment (Turner et al. 2014; Shogren et al. 2016; Jo et al. 2019). These classes broadly include encapsulated eNA that is still located within dying or dead cells that are no longer associated with whole organisms or within cell-liberated organelles, unencapsulated eNA and eNA adsorbed to co-occurring ionic substrates, such as clay particles. The fate of eNA largely refers to what happens to eNA once the nucleic acid is no longer associated with an intact, normally functioning cell. Molecular degradation, along with preservation, are key facets of eNA fate that influence the capture and detection of eNA. Transport is the physical displacement of eNA from its point of origin. It is important to consider transport processes regarding both the species and the environment being studied, as it will likely affect capture strategies and study conclusions. This review will synthesise results from studies relating to *Dreissena* and other species with similar life histories as they relate to the ecology of eNA for the purpose determining the best sampling methods for *Dreissena* eNA detections.

Materials and methods

We sought for relevant reports of *Dreissena* eNA studies via searches of both Google Scholar and Web of Science (Core Collection Database; Clarivate, Philadelphia, PA, USA). Each database was last searched on 13 December 2024. Search terms included each of the following, limited to key terms in the titles: "*Dreissena*

Environmental DNA", "*Dreissena* eDNA", "*Dreissena bugensis* eDNA", "*Dreissena bugensis* Environmental DNA", "Dreissenid eDNA", "Dreissenid Environmental DNA", "*Dreissena polymorpha* eDNA", "*Dreissena polymorpha* Environmental DNA", "Quagga eDNA", "Zebra eDNA", "Quagga Environmental DNA", "Zebra Environmental DNA", "eDNA transport", "Environmental DNA transport", "eDNA particle size distribution", "eDNA fate". These search terms resulted in 89 non-redundant papers. We did not review papers that were in a marine or terrestrial environment (n = 10), papers that did not focus on *Dreissena* (n = 52) or papers that did not either directly or indirectly provide information for the ecology of eDNA (n = 22). We did not include meeting abstracts (n = 1) or reports/grey literature (n = 4). Some of the manuscripts that we culled were not relevant for multiple of the above reasons. If a publication had seemingly relevant literature cited that did not appear in our literature search, that additional literature was reviewed (n = 3). Each publication, identified as potentially relevant, was reviewed to ascertain that it contained results and observations pertinent to *Dreissena* eNA ecology, the capture of *Dreissena* eNA. In cases where multiple papers provided results or observations pertinent to the same facet of *Dreissena* eNA surveys, we included all these observation and findings in this review. Instances where study outcomes and observations appear to conflict or would provide contrasting guidance are noted. In some cases, these reports led us to search for and review additional papers that did not directly address eNA, but instead provided key information about *Dreissena* natural history or the hydrology of different natural aquatic ecosystems. These papers were included to provide additional explanation or context to the results drawn by the reviewed *Dreissena* papers, but were not explicitly included in the review and, thus, are not an exhaustive list of the papers that deal with the ecology of eNA or the life history of *Dreissena*.

Results

A total of 13 publications (Suppl. material 1) were identified as being pertinent to the topic of designing *Dreissena* eNA monitoring efforts that focus on detecting early stage, incipient quagga or zebra mussel invasions. Of the 13 publications, 12 deal specifically with eDNA and one covers the use of eRNA. Key observations and information related to the stated focus of this review from all 13 articles are described or cited in the Results section.

Origins of *Dreissena* eNA

There are no publications directly addressing the origins of *Dreissena* eNA, but several publications provide indirect evidence regarding some likely sources of *Dreissena* eNA. *Dreissena* eNA in natural settings would be produced and deposited through means similar to nearly all other animals, as stated previously (Barnes and Turner 2016). For example, during cold season sampling of a lake for *Dreissena* eDNA, including a period where the surface water was frozen, Amberg et al. (2019) detected *Dreissena* eDNA near the lake bottom, but not at the surface (just below the ice) and attributed this eDNA to non-buoyant faeces and pseudo-faeces.

Broadcast gametes and planktonic juvenile stages appear to be substantial seasonal contributors to the *Dreissena* eNA pool. This has been directly observed in species with similar life histories to *Dreissena*, such as the freshwater mussel

Margaritifera margaritifera. Wacker et al. (2019) observed higher eDNA detection rates for during periods when larvae were being expelled from adult mussels. In Black Sea bream, *Acanthopagrus schlegelii*, which have a pelagic egg and larval stage, researchers found an increase of 1.5 to 3 times in *A. schlegelii* eDNA detections during the species' spawning season and at further distances from the source (Sasano et al. 2022).

For *Dreissena*, Sepulveda et al. (2019) obtained 50% of positive *D. bugensis* eDNA samples in the recently invaded Tiber Reservoir, in Montana, USA, during a warmer period in July, whereas surveys during cooler periods before and after July each had positive detections in just 0.03% of their samples. The authors attributed the increased incidence of positive samples to *Dreissena* reproduction and rising concentrations of *Dreissena* eDNA. Rounds et al. (2024) conducted a series of eDNA surveys for multiple species, including *D. polymorpha*, over 16 – 24 months on a set of 20 northern US lakes. As observed with other studies, *Dreissena* eDNA detection frequency amongst samples was substantially higher during the warm summer months when spawning and juvenile development would be occurring.

During a four-week study of *Dreissena* eDNA-bearing particle sizes in Canyon Lake, TX, USA, Plate (2023) recorded a temporary increase in both eDNA concentrations and a particle-size class that would likely include gametes and juveniles, suggesting that a spawning event had likely caused the eDNA spike. Increased *Dreissena* eDNA and detection rates associated with increased water temperature (and likely *Dreissena* reproduction) have been observed, to varying degrees, in several other studies (Peñarrubia et al. 2016; Gingera et al. 2017; Amberg et al. 2019; Sepulveda et al. 2019; Peixoto 2024; Rounds et al. 2024).

Mass mortality is likely another major source of *Dreissena* eNA. Both extreme warm and cold temperatures, the latter in combination with lowered water levels and open air exposure, can kill adult and juvenile mussels (McMahon 1996; Leuven et al. 2014; White et al. 2015). We hypothesise that such lethal environmental conditions may result in a positive flux of eNA as large amounts of previously encased and highly localised eNA escape decaying cells. Molluscicide treatments would likely have the same effect. Another source of mortality in *Dreissena* is predation. While there are no published studies that address this specifically for *Dreissena*, there are documented cases of using predator waste to survey prey (Merkes et al. 2014; Boyer et al. 2015; Guilfoyle et al. 2017; Nørgaard et al. 2021). Substantially increased predation rates, such as may occur with the arrival of migratory predators, could increase *Dreissena* eDNA in a system. In the Great Lakes area, migratory waterfowl that prey on *Dreissena* include the greater and lesser scaup (*Aythya marila* and *A. affinis*), common golden eye (*Bucephala clangula*), bufflehead (*Bucephala albeola*), long-tailed duck (*Clangula hyemalis*) and white-winged scoter (*Melanitta deglandi*) (Mitchell et al. 2000).

State of *Dreissena* eNA

One approach that has been utilised to partially characterise the state of eNA is particle size distribution (PSD) (Turner et al. 2014; Wilcox et al. 2015; Jo et al. 2019). PSD describes the relative proportion of target species eNA-bearing particles found within different size classes, as determined by filtration of samples through filters with varying pore sizes and follow up qPCR to quantify eNA of the

target species. PSD studies can provide evidence as to whether eNA from a target species is largely comprised of unencapsulated free strands of DNA, organelle encapsulated material or whole microscopic organisms. The PSD of the DNA will determine the size, buoyancy and tendency to bind or aggregate to other materials. Thus, the PSD changes how DNA moves through the environment and whether it behaves like a solute or a particle, its settling rate (Turner et al. 2014), the optimum sampling method (Jo et al. 2019) and the distance travelled from deposition (Zhao et al. 2021; Brandão-Dias et al. 2023). This creates challenges when designing a model to locate populations, based on eDNA detections, as well as for determining optimal locations to sample.

Plate (2023) conducted a PSD study of *D. polymorpha* eDNA in Canyon Lake, TX, US over a four-week period in the late summer and early autumn of 2021. In this study, the majority of *Dreissena*-eDNA bearing particles were found in 0.2 –1.0 μm (41% ± 5%) and ≥ 12 μm (33% ± 5%) size classes, followed in rank order by the 1.0 –3.0 μm, 3.0 –5.0 μm, 5.0 –8.0 μm and 8.0 – 12.0 μm particle size classes. These results indicated that important constituents of the *D. polymorpha* eDNA pool included eDNA associated with larger particles, which could include whole ova and juveniles, based on length and diameter spermatozoa might be expected to occur to some degree in all smaller size classes, except 0–0.2 μm (Ram et al. 1996). Plate (2023) also noted that the relative portions of *D. polymorpha* eDNA size classes varied over both time and space.

Fate of *Dreissena* eNA

Generally, eNA is relatively stable at low water temperatures (e.g. 4–5 °C), with increasingly rapid decay as water temperatures climb (Strickler et al. 2015; Jo et al. 2017; Lance et al. 2017). Though there is an increase in eNA molecular thermokinetics with increasing temperature, the primary factor driving this phenomenon is likely increased microbial activity and the breakdown of nucleic acids by bacterial exonucleases (Nielsen et al. 2007; Strickler et al. 2015; Lance et al. 2017). Nucleic acids can be a source of carbon, phosphorus or nitrogen to microbes that have metabolic pathways that allow for it (Jørgensen and Jacobsen 1996; Beattie et al. 2023). It should be noted, however, that the links amongst temperature, microbial activity and eDNA degradation have not been observed in all studies (Barnes et al. 2014; Tsuji et al. 2017) and are likely influenced by a complexity of factors in natural environments. UV-B radiation damages nucleic acid strands and exposure to UV-B may increase eNA degradation rates, though studies of UV-B effects on eDNA have been mixed (Pilliod et al. 2014; Strickler et al. 2015; Andruszkiewicz et al. 2017; Mächler et al. 2018). Under natural conditions, where solar irradiance is the primary driver of water temperature, it may be challenging to separate effects of water temperature and UV-B exposure on eNA degradation (Pilliod et al. 2014).

Both eDNA and eRNA can adsorb various components of sediment and suspended sediment, including clays, humics and silicates (Goring and Bartholomew 1952; Lorenz et al. 1981; Yu et al. 2013; Biondi et al. 2017). When adsorbed to sediments, eNA is protected from nucleases and environmental conditions that contribute to molecular decay and degradation (Cai et al. 2008; Pietramellara et al. 2009; Eichmiller et al. 2016; Chatterjee et al. 2023). The pH of a system may directly influence the molecular decay of eNA, the eNA adsorption to sediment and soil and the activity of nucleases, but the presence and direction of this effect

varies across studies (Strickler et al. 2015; Lance et al. 2017; Seymour et al. 2018; Kagzi et al. 2022).

The fate of *Dreissena* eNA has been studied or at least alluded to in a few studies. Marshall et al. (2021) studied the decay in concentration for six different eDNA and eRNA loci derived from four different genes. These loci are found within the 18S structural RNA gene and protein-encoding H2B gene from the nuclear genome and the 16S structural RNA gene and protein-encoding COX I gene from the mitochondrial genome. Two amplicons of differing length were assayed for eNA from both the 16S and H2B genes. The results indicated that *Dreissena* eRNA decayed at a more rapid rate than *Dreissena* eDNA, with decay half-lives in the approximate ranges of 9.5–12.5 hours and 14.5–18.5 hours, respectively. The lower bound on the eRNA half-life estimate was somewhat truncated, because the H2B eRNA decayed below detectable levels too rapidly for effective measurement. However, the eRNA derived from the two structural RNAs (16S and 18S) occurred at such high initial concentrations that the eRNA loci were both detected at much higher relative frequency than their corresponding eDNA. As has been observed for *Dreissena* in other studies (Blackman et al. 2020), the eDNA and eRNA loci studied by Marshall et al. (2021) exhibited exponential decay patterns typical of eDNA. Over the course of the study, the ratio of eRNA:eDNA decreased due to the more rapid decay of eRNA.

Shogren et al. (2019) explored the role of *Dreissena* eDNA removal though natural sedimentation and other eDNA trapping dynamics in a Danish river system. The authors estimated that as much as 50 – 80% of *Dreissena* eDNA was lost from the water column through processes other than molecular decay and degradation. Amberg et al. (2019) studied *Dreissena* eDNA in a lotic system and found that eDNA was present in sediment across all seasons. As described earlier, eNA that is adsorbed to sediment constituents is expected to exhibit prolonged persistence relative to eNA in the water column. Peixoto (2024) observed a weak trend for *Dreissena* eDNA to be more concentrated over gravel substrates than silt substrates in several different types of waterbodies in northern England, though no such differences were observed between these and boulder or sand substrates (or between boulders and sand).

Transport of *Dreissena* eNA

In many lentic systems, dispersion and transport of eNA from a point source may be limited to distances from practically zero to a few tens of metres (Dunker et al. 2016; Harper et al. 2019; Li et al. 2019). However, phenomena such as currents and seasonal turnover may create complex eNA transport dynamics and result in greater dispersal distances. For example, eNA dispersion in ponds may be impacted by winds, undercurrents and physical barriers (Mayne et al. 2024). In larger systems, such as Lake Michigan, surface currents move counter clockwise in the spring and clockwise in the summer (Beletsky et al. 2017), impacting *Dreissena* veliger transport patterns (and, thus, eNA transport patterns). Many temperate region lakes develop seasonal thermoclines that stratify biological communities by depth, which then stratifies a taxon's eNA concentrations along depth and temperature gradients (Lawson Handley et al. 2019; Littlefair et al. 2021). These stratified layers do not mix during the winter and summer months, but during the process of biannual lake turnovers or inversions, the water, organisms and eNA

may become more homogeneously distributed. Near the surface of lentic systems, wind may also be a major factor in the movement and dispersion of buoyant particles (Sun et al. 2020).

Larson et al. (2022) were able to link patterns in summer lake currents to patterns in veliger and eDNA concentrations and, as such, provide support for a hypothesised mechanism of *Dreissena* colonisation of islands within western Lake Superior (CA/USA). Winds and storms can also influence the transport of *Dreissena* eNA in lentic environments. For example, *Dreissena* veligers suspended near the water surface may be transported along with wind–driven, near–surface currents. In a study on Lake Erie (USA/CA), Marshall and Stepien (2019) found that the eDNA of *D. polymorpha* was more prevalent than the eDNA of *D. bugensis* at sites where the former had greater biomass, except when a strong westward wind appeared to drive a current containing a higher concentration of *D. bugensis* eDNA into the locale. Notably, the potential effects of thermal stratification on vertical distribution of *Dreissena* eNA have not been studied.

In the only published comparison of sampling for *Dreissena* eDNA at different depths, Amberg et al. (2019) found seasonal differences in *D. polymorpha* eDNA concentrations and detection frequencies. While the study obtained *Dreissena* eDNA detections at all four sampling depths (1, 2, 4 and 6 m) in Lake Le Homme Dieu and Maple Lake in Minnesota, USA, an increase in eDNA concentrations with depth was found in Lake Le Homme Dieu, but not Maple Lake. As part of their method development for this study, Amberg et al. (2019) also found that *Dreissena* eDNA was detectable in surface, mid-column and near-bottom water samples taken mid-summer from Lake Minnetonka, MN, USA, but concentrations appeared to be comparatively higher at the surface and near-bottom than at mid-column. They also noted that *Dreissena* eDNA concentrations could be unexpectedly high in reaches of soft sediment, which are generally considered poor or less optimal for *Dreissena* settlement. The authors posited that this phenomenon resulted from *Dreissena* eDNA accumulation in reaches of the lake bottom where sediment and detritus accumulate.

Lotic systems are typically dominated by a strong directional current and more energetic interaction between water flow and bottom substrates and eNA has been detected many kilometres from source populations in such systems (Deiner and Altermatt 2014). The extent to which eNA is transported beyond its source will be a function of eNA concentrations at the source, stream volume and stream flow rates. For example, Fremier et al. (2019) observed that in steeper, faster flowing and more energetic streams, eDNA was detected further from the deposition location than in lower grade streams. The authors posited two possible mechanisms that might result in the observed pattern: the higher velocity of steeper streams keeps eDNA suspended or the higher turbulence of higher velocity streams re-suspends eDNA from the benthos. In a caged fish study simulating a lotic system, Wood et al. (2020) found that, due to eDNA dispersing from its source in a directional plume, eDNA detection might be low or negligible in close proximity to the source, except at a narrow span directly downstream from that source. The substrate of a system may also influence eNA transport. For example, finer substrates appear to retain a greater portion of overall eDNA than larger substrates (Aubeneau et al. 2014; Jerde et al. 2016). Aquatic biofilms, which are microbial consortia that adhere together within the slimy layer of various substrates, have also been found to both trap and remove eNA, in both running and still waters (Shogren et al. 2018; Wood et al. 2020). Several models have been developed for

understanding or predicting the transport of eDNA in lotic systems or for more robust interpretation of data from such systems (Jane et al. 2015; Sansom and Sassoubre 2017; Shogren et al. 2017; Carraro et al. 2018; Cerco et al. 2018; Nevers et al. 2020; Wood et al. 2020, 2021; Pont 2024).

In a recently invaded lotic system, Shogren et al. (2019) were able to distinguish distance from the source colony for *D. polymorpha* eDNA in stretches of the study river where the authors' model indicated that eDNA production outpaced eDNA decay and removal. Sampling for *Dreissena* eDNA in this study was conducted in May in Denmark when water temperatures were below those typical for reproduction and development of juveniles (12–18 °C), minimising the influences of these sources of eDNA on the study. *D. polymorpha* eDNA was not detected in the immediate downstream reach (0–163 m) of the furthest extent of *Dreissena* establishment, but was detectable downstream of that colony for up to several kilometres. eDNA samples collected closer to the source had significantly narrower ranges (i.e. lower variance) in eDNA copy number estimates than samples collected further downstream. The authors also noted a trend towards higher eDNA concentrations mid-stream than along the shoreline. The study further observed significant positive correlations between eDNA concentration and both soluble nutrient (NH_3, PO_4^{3-}) concentrations and water velocity. No explanations for the correlation between nutrients, water velocity and *Dreissena* eDNA concentration were attempted, though water turbulence and velocity may keep eDNA particles suspended or re-suspend eDNA particles that have sunk to the sediment layer (Fremier et al. 2019; Preece et al. 2021).

In a study of *D. bugensis* in the River Wraysbury in south-eastern England (UK), Blackman et al. (2020) observed significant negative correlations between eDNA concentration and distance from source colonies, but only if samples from just below the source were removed from the dataset. As with Shogren et al. (2019), the immediate downstream reach of the watercourse below the primary source of *Dreissena* eDNA had much lower eDNA concentrations than might be expected. At six *D. polymorpha*-infested lakes in TX, US, Whitehead (2023) observed detections of *D. polymorpha* eDNA as far downstream as 27 river km, but with no consistent trend of decreasing eDNA concentrations with distance. The study also found no significant statistical relationship amongst observed eDNA concentrations and specific conductance (or highly correlated turbidity), water temperature, dissolved oxygen, discharge rate or streambed substrate. The results of this study, however, may have been somewhat confounded by incomplete knowledge of whether adult zebra mussel populations were established within the stretches of river between sampling locations.

Discussion

The strategic implications for *Dreissena* eNA monitoring that arise from the material covered in this review are discussed in detail below. These implications lead to a number of *Dreissena* eNA monitoring recommendations, which are also discussed below, as well as summarised in Table 1.

Strategic implications: Seasonal monitoring

There are several *Dreissena* eNA detections probabilities that are affected by temperature and season. Spawning and the subsequent formation of the floating trochophore and veliger stages are seasonal, thus strategic selection of eNA survey

Table 1. Summary of general sampling recommendations for detecting *Dreissena* eNA in an early invasion. These recommendations include both lentic and lotic environments. These suggestions do not include strategies for locating early invasions.

Lentic		General Detection Strategies
	Location	Just above the thermocline to maximise veliger detections.
		Surface samples for veliger detections.
		Benthic samples in seasons with no veligers.
		Consideration of water current and wind patterns.
		Near boat ramps or other vectors of transport.
	Timing	Sampling during lake turnover.
		Sample during spawning and veliger presence.
		Periods of high mortality.
Lotic		
	Location	Areas of high energy such as riffles.
		Near boat ramps or other vectors of transport.
		Sampling within the watercourse as opposed to bankside.
	Timing	Sample during spawning and veliger presence.
		Periods of high mortality.

periods may substantially enhance detection probabilities (Sepulveda et al. 2019; Plate 2023; Peixoto 2024). Conversely, when attempting to discern the actual locations of colonies or patches of adult *Dreissena*, sampling outside the spawning season may allow for more spatially precise survey outcomes. There are no eNA methods currently available to differentiate between veligers and adult Dreissenids; however, it is possible that assays that monitor differences in gene expression between veligers and adult mussels may one day be applied to eNA to differentiate signals from established adult populations and floating larval stages. Until then, the reliance on seasonal sampling and visual confirmation is required. In cases where general detection in lakes is an objective and the system is seasonally stratified, sampling just above the thermocline may "empower" surveys by taking advantage of eNA associated with concentrations of veligers (should *Dreissena* have colonised the system). Sampling during lake turnover may also increase the detection probability without needing to increase the sampling efforts by sampling below the thermocline. Seasonal shifts in water temperature influence food availability and, thus, may impact the rate at which faeces and pseudo-faeces are produced. The amount of pseudo-faeces produced by *Dreissena* is dependent on the quantity of suspended particles being ingested, which is directly related to filtration rate and which increases with temperature within their zone of optimum temperature (Diggins 2001).

Extreme climatic periods with different seasons may impact *Dreissena* mortality, which would impact *Dreissena* eNA concentrations substantially. When *Dreissena* are exposed to acute (30 °C–32 °C) or chronic (accumulated degree hours > 25 °C) lethal temperatures, mass mortality may occur (White et al. 2015). Additionally, the spring and summer arrival of migratory molluscivores, such as many waterfowl, may increase rates of *Dreissena* mortality.

As an example of seasonally-strategic sampling, Gingera et al. (2017) recommended sampling for *Dreissena* in the late summer or early autumn in Manitoba, CA for several reasons. One, this period would be in the later part of the breeding period when adult mussel biomass would be close to its peak and when the juvenile

stages would still be present in the system, maximising *Dreissena* eDNA detection chances. Two, conducting *Dreissena* eDNA monitoring when detection probabilities are at a maximum enhances confidence that surveys resulting in zero detections reflect a true absence of *Dreissena*. Three, for very cold regions like Manitoba, which have minimal mussel activity during late autumn and winter, late season eDNA monitoring would provide the best indication of where *Dreissena* populations might be found the following spring.

Strategic implications: Sampling depth and points

In lentic systems, adult *D. polymorpha* tend to be found in the epilimnion, particularly in littoral zones with hard substrates for settling. However, adult *D. bugensis* may occur across the epilimnion, metalimnion and hypolimnion reaches of lakes, including reaches with primarily soft substrates (Melis et al. 2010; Karatayev et al. 2015). *Dreissena* veligers can be found in the epilimnion and metalimnion, but tend to be more abundant in the epilimnion (Bowen et al. 2018; Kirkendall et al. 2021) and also more abundant in the relatively deep epilimnion reaches near thermoclines (Reid et al. 2011; Churchill 2013). There are a number of advantages to sampling water at the surface or near-surface for eNA compared to sampling near the bottom of the system or from the sediment (Pawlowski et al. 2022), including a wider variety of well-established protocols, fewer equipment limitations (e.g. availability of Niskin bottles, Ponar grabs or pumps with sufficient draw for depth sampling) and typically reduced inhibitor concentrations (relative to sediment sampling). When monitoring for *Dreissena* eNA in lentic systems, sampling surface waters for *Dreissena* eNA or sampling near thermoclines during periods of juvenile development and dispersal, would appear to be sufficiently effective in most cases. In some scenarios, sampling water near the bottom of a lake or pond might represent a further investment in vigilance if surface water samples are found to be entirely or largely free of *Dreissena* eNA detections. Potential benthic accumulation zones, when known, might represent high value near-bottom/bottom sampling points (Amberg et al. 2019).

In lotic systems, reaches with higher turbulence, such as riffles, may represent key locations for *Dreissena* eDNA sampling. Water turbulence appears to keep eDNA suspended in the water column (Fremier et al. 2019), whereas less energetic reaches (e.g. runs and pools) allow for greater proportional eDNA settlement to the benthos. This factor may be particularly impactful during periods when floating *Dreissena* gametes and juveniles are not present. It is conceivable that strategic sampling of surface waters in different kinds of watercourse features could be employed to achieve different aims, such as early detection over broad lengths of a stream course or narrowing down source population locations.

Strategic implications: Geographic siting and spacing

Given that *Dreissena* are readily transported by watercraft, watercraft trailers and float planes, it seems that boat ramps, docks and other structures that accommodate these vectors would be logical sites for *Dreissena* eNA monitoring of locales that are currently free of these invaders (Gingera et al. 2017). Formal coupling of *Dreissena* eNA monitoring with models of site introduction risk, habitat suitability and system hydrology would be interesting and potentially very powerful strategies

for discovering establishment of new *Dreissena* populations (Bossenbroek et al. 2001; Carraro et al. 2017; Barnes and Patiño 2020; Robertson et al. 2020).

The optimal siting of water sampling points for *Dreissena* eNA monitoring in lentic systems will be largely determined by a combination of waterbody area, volume and depth. However, strategic sample plans may reduce the effort required, which would be key for large lentic bodies. For example, sampling surface waters during periods when water temperatures are conducive to spawning and buoyant eNA-bearing items (i.e. gametes, veligers) are likely to be present may preclude the need to sample near the benthos, along with the accompanying technical challenges of doing so. Understanding currents and wind patterns could allow for selective strategic sampling of reaches of lentic systems where eNA would be transported for potential hot spots or accumulate (Marshall and Stepien 2019; Larson et al. 2022). Prior efforts in similar systems and with similar taxa could provide basic guidance for designing survey methods, while pilot studies, when possible, are generally highly useful and recommended (Goldberg et al. 2016).

In lotic systems, the spacing of sampling points will also depend on system area and volume, but with the added factor of flow rates or discharge (Jo and Yamanaka 2022). Again, sampling during periods when buoyant eNA items (i.e. gametes, veligers) are present minimises depth concerns for survey designs, as would focusing sampling on reaches with higher turbulence. Thus, streams and rivers with relatively high turbulence may allow for less-closely spaced monitoring points. However, if such conditions only occur during periods of peak system volume (e.g. floods), then the advantages of turbulence and flow rate may be offset by greater eNA dilution and, often, increased water turbidity that reduces sample volume (when filtering) or increases PCR inhibition.

In addition to targeting lotic features with relatively high turbulence for sampling, sampling from within the watercourse (as compared to bankside) should enhance *Dreissena* eNA detection probabilities. This is because eNA disperses from source as a plume in lotic systems and unless the source is bankside and samples are collected from the same bank, *Dreissena* eNA may not be present (Wood et al. 2020). As noted for lentic systems, survey designs established for similar systems or pilot studies may help provide information for optimal spacing of eNA survey points along the system. The approaches or models established for predicting or interpreting eDNA transport in lotic systems noted previously may also be useful.

Strategic implications: Locating dreissena populations

Initial surveys for *Dreissena* eNA may be quite broad, with the goal of simply determining if the mussels are present somewhere in the system. For example, in surveys for *Dreissena* in the Ticino River watershed in Italy and Switzerland, Capelli et al. (2023) pooled water samples from multiple sites within different lake basins to reduce the number of samples and the time and cost for initial eDNA surveys. This approach, however, risks diluting positive detections with negative detections, thereby decreasing the sensitivity of the sampling effort.

However, once *Dreissena* eNA is detected in a system, the spatial window within which the source(s) of the eNA could be determined may be reduced by a variety of strategic options, which could be employed singly or in combination. For example, after testing water samples with *Dreissena* eNA assays targeting a highly abundant gene (e.g. 18S) in order to establish that one or both of the invaders

are likely present, re-testing the samples for a less abundant gene (e.g. H2B) may narrow the potential range within which the source population may be found, because the less abundant gene is more likely to decay below detection values closer to the source population than the more abundant gene (Marshall et al. 2021, 2022; Feist et al. 2022). Another strategic option would be to follow up initial surveys that were conducted during periods of peak *Dreissena* eNA abundance and distribution (e.g. during spawning, veliger dispersal, water inversion in stratified lakes) with additional samples collected during times of reduced eNA abundance and distribution (e.g. prior to or after spawning and veliger dispersal, periods of low water flow or turbulence). This strategy assumes that, during periods of decreased eNA production, detectable concentrations of eNA would be found within a comparatively reduced spatial window to the source population in lentic systems and comparatively shorter downstream range of the source in lotic systems. When available, this strategy would benefit from archived multi-year datasets on daily, weekly or monthly water temperatures, depths and flows for the system of interest. In lotic systems, once a reach is found to have several positive samples, follow-up surveys with comparatively closely-space sampling along each bank could delineate the point(s) where an eNA plume ceases to be detected, indicating that the plume source is either upstream near that point or proximate to that point for a near-bank source. Pairing bank-side samples with mid-stream samples could also provide an effective means of minimising the area within which the source population of *Dreissena* is likely located.

There are several caveats to these approaches. One, due to the inherent patchiness and typically very low concentrations of eNA in aquatic systems, stochastic non-detections within the range of the eNA detection should generally be expected when considering survey results. Determining if non-detections are due to insufficient sampling effort or true absence of *Dreissena* can be determined in part with occupancy or occurrence models (Burian et al. 2021). Two, the presence of more than one eNA source (e.g. multiple dispersed colonies or clusters) in the lotic system could provide relatively complex survey results that are more difficult to interpret. While our review of strategic eNA monitoring for *Dreissena* is primarily focused on detecting new invasions, where multiple sources are less likely, patterns of increasing and decreasing numbers of positive samples or increasing and decreasing eNA concentrations in positive samples as a survey moves upstream may reveal the presence of multiple sources. Three, as noted above, eNA concentrations between sampling points can be highly variable over small spatial scales, being influenced in complex ways by substrate, microbial abundance and activity, UV exposure, PCR inhibitor concentrations and classes etc. PCR/qPCR detection, however, is determined by threshold concentrations of DNA or RNA. Unless eNA concentrations (post NA extraction) are hovering around that threshold, detection will be insensitive to the shifts in eNA concentrations over those small spatial scales. Attempts to hone in on eNA sources using patterns in estimated eNA concentrations, as opposed to patterns of PCR/qPCR hits and misses, will be more susceptible to being misled by the aforementioned variance in eNA concentrations amongst sampling points. Four, at the point where eDNA detections cease, it might be tempting to assume that the source population is continued sampling upstream of sites where eNA is no longer detected would help guard against misinterpreting localised outcomes that are not true indicators of overall eNA patterns within the waterbody.

Strategic implications: Sampling effort

An aspect of early invasion monitoring that is outside the scope of this study is the sampling effort required (i.e. the number of samples taken per waterbody) in early infected waterbodies to have a positive detection. This concern can be addressed using occupancy or occurrence models. Occupancy models provide quantitative likelihoods for the occurrence of false positives and false negatives and provide guidance on the confidence with which eNA results may be viewed (Burian et al. 2021), including for surveys resulting in zero detections. While there have been no studies describing occupancy modelling for *Dreissena* eNA monitoring, Winder et al. (2022) developed occupancy models for plankton tow net surveys for *Dreissena* veligers in different-sized waterbodies. Additionally, eDNA survey occupancy models have been described for several species, including, but not limited to the endangered *Percina rex*, the near threatened *Ornithorhynchus anatinus*, the invasive *Hypophthalmichthys nobilis* and *H. molitrix* and the fish pathogen *Tetracapsuloides bryosalmonae* and fish *Oncorhynchus nerka* (Mize et al. 2019; Strickland and Roberts 2019; Sepulveda et al. 2021; McColl-Gausden et al. 2024). Given that the presence of veligers will generally be a crucial factor in *Dreissena* eNA monitoring, combining aspects of the plankton tow model with the occupancy model approaches implemented for eDNA data might prove highly useful. Thus, we anticipate that any early-detection surveys or monitoring programmes that are developed for *Dreissena* will at least explore and, likely, implement occupancy modelling.

Quantifying dreissena populations from eDNA

Our review of the practical considerations that would underlie strategic *Dreissena* eNA monitoring for incipient populations has focused solely on factors that would impact detection, primarily of early-stage invasions comprised of discrete, small populations. However, a common question that arises for nearly all eNA efforts is whether eNA monitoring can provide inference on target taxon population numbers or biomass. The interplay between the size of the invasive population and eNA levels at a sampling location is extremely complex, with a strong dependency on eNA production rates for different classes of eNA, lotic flow rates or lentic currents, system volume, retention of eNA by substrates and re-suspension and eNA decay – all of which may show strong temporal (e.g. seasonal) and between-site variance. Not surprisingly, as with many other taxa in which correlations between eDNA concentrations and population size have been studied, the outlook for *Dreissena* has been mixed (De Ventura et al. 2016; Amberg et al. 2019; Shogren et al. 2019; Blackman et al. 2020). The most useful approach would be to test eNA–population size relationships via pilot studies in the system of interest or, perhaps, a surrogate system, but this may not be practical, especially where early detection in currently non-invaded systems is the objective.

Conclusions and future research

Though the body of literature surrounding *Dreissena* eNA is robust compared to that of most other species, the variety of aquatic systems in which *Dreissena* eNA surveys may be employed, the inherent complexity of eNA ecology and different

objectives for *Dreissena* eNA surveys make concrete guidelines for such surveys difficult to establish. Nonetheless, there are a few basic considerations that are likely helpful. One, taking season into account is crucial for *Dreissena* eNA surveys, particularly whether it benefits the objective of the survey to capture eNA associated with the buoyant *Dreissena* gametes and juveniles. Two, in most cases sampling at or near the surface of the waterbody will be sufficient for robust *Dreissena* eNA detection probabilities. Three, sampling in lentic systems may benefit from the consideration of currents and wind patterns. Four, it is likely that focusing water sampling in lotic systems on mid-stream reaches with higher turbulence will increase *Dreissena* eNA detection probabilities.

Our review has highlighted several future research questions and topics that would benefit *Dreissena* eNA surveys and monitoring programmes. These questions and topics are listed below:

1. For lentic systems, studies of dispersion of *Dreissena* eNA from discrete adult populations or infested reaches in both the absence of currents and in systems with strong or persistent wind patterns is needed to improve our understanding of transport in these systems. Subsequent studies of current- and wind-driven dispersion of *Dreissena* eNA may serve to provide information for strategically optimised monitoring designs.
2. We have proposed that, in lotic systems, sampling within turbulent stretches of the stream may enhance detection, but this has not been studied or demonstrated for *Dreissena* eNA. Studies that encompass tests of detection rates from different classes of eNA at varying distances from sources and from within different lotic features would be ideal.
3. We have identified strategic seasonal sampling as the best way to confirm spawning; however, if eNA assays were developed that identified differences in gene expression between adults and juvenile individuals, molecular methods could be used to confirm spawning.

Future capabilities for eNA-based monitoring to quickly identify invasive populations of *Dreissena* and other aquatic invasive species are continually evolving (e.g. Marshall et al. (2021); George et al. (2024); Miller et al. (2024); Whitehead et al. (2024)). The strategic considerations outlined here and the future research needs identified will be key to realising the full potential of these capabilities for meeting the invasive *Dreissena* challenge. Combined with growing public awareness of the invasive species threat and participation in prevention programmes, these capabilities may yet result in natural resource managers and stakeholders gaining leverage on *Dreissena* and other invasive species challenges.

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Additional information

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The authors have declared that no competing interests exist.

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Use of AI

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Author contributions

Conceptualization: RL. Funding acquisition: RL. Supervision: RL. Writing – original draft: MPM. Writing – review and editing: RL, MPM.

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

List of manuscripts_Monitoring for incipient dreissenid mussel invasions using environmental DNA and RNA

Authors: Madeleine Malmfeldt, Richard Lance

Data type: docx

Explanation note: List of 13 published accounts of *Dreissena* environmental DNA (eDNA) and environmental RNA (eRNA) studies that provide direct observations or useful inference about the ecology of Dreissena eDNA and eRNA.

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